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Letter to the Editor

Infant C677T Mutation in *MTHFR*, Maternal Periconceptional Vitamin Use, and Risk of Nonsyndromic Cleft Lip†

To the Editor:

Cleft lip with or without cleft palate (CL/P) has a prevalence at birth between 1/500–1/1000 and is one of the most common malformations among live births. It is well known that both environmental and genetic factors play significant roles in the development of CL/P [Wyszynski and Beaty, 1996; Wyszynski et al., 1996; Schutte and Murray, 1999]. Among the environmental effects, there is conflicting evidence as to whether multivitamin supplementation before pregnancy or early in pregnancy reduces risk of oral clefts [Czeizel, 1993; Czeizel and Hirschberg, 1997; Hayes et al., 1996; Shaw et al., 1995; Tolarová and Harris, 1995; Werler et al., 1999].

Recently, Shaw et al. [1998] reported a large population-based, case-control study of CL/P newborns among a cohort of 1987 to 1989 California births. Interviews were administered to the mothers of cases and controls asking about the types of vitamin supplements they used (prenatal vitamins, multivitamins, vitamin A, folic acid, and other types). Cases and controls were genotyped for the C677T single nucleotide polymorphism (SNP) of the 5,10-methylenetetrahydrofoloate reductase (*MTHFR*) gene. In the statistical analysis, risk of clefting was evaluated for subjects having the TT or the CT genotypes compared with the homozygous CC genotype as the referent group. No significant differences were found for these comparisons of the *MTHFR* genotypes stratified within groups by maternal use versus nonuse of periconceptual multivitamins. The authors conclude that their results "do not indicate increased risk for CLP among infants homozygous for the C677T genotype, nor do they indicate an interaction between infant C677T genotype and maternal multivitamin use on the occurrence of CL/P."

We noted, however, that statistical comparisons were made only between genotype groups for subjects who had the same history of periconceptual multivitamin use (i.e., either used or did not use multivitamins). The main effects of multivitamin use or genotype were not formally estimated, nor was the possibility of a genotype by environment (G×E) interaction evaluated. Therefore, we reanalyzed the data presented in Table II of Shaw et al. [1998] using logistic regression based on a single referent group to address these questions, as shown in our Table I. Odds ratios (OR) and *P*-values were calculated for main effects and G×E interaction, and Cornfield's 95% confidence intervals were obtained using the LOGISTIC procedure of the SAS software [SAS Institute, 1996]. Mantel-Haenzel pooled ORs and estimates of attributable fraction were calculated with the software IC2X2 [Thomas and Gart, 1992]. It is clear from inspection of the odds ratios shown in Table I that maternal nonuse of vitamins is associated with increased risk of clefting, with statistically significant differences found for two of the three genotypes. When tested in the logistic regression model, the overall effect of vitamin use (adjusted for *MTHFR* genotype) is statistically significant ($P = 0.03$, Mantel-Haenzel pooled OR: 2.30, 95% CI: 1.56–3.40), with increased oral cleft risk associated with nonuse of vitamins (OR: 4.0, 95% CI: 1.15–14.0). CL/P risk appears to decrease slightly between the CC and TT genotypes for vitamin users and increase for nonusers for these genotypes, but neither the *MTHFR* genotype main effect (adjusted for vitamin use) was statistically significant ($P = 0.26$), nor was there significant evidence of a G×E interaction ($P = 0.38$). The attributable fraction for the absence of multivitamin use was 56% among cases and 33% (95% CI: 19.8–46.7) among the total population, assuming that 59% of the population is exposed.

Our reanalysis of the data presented by Shaw et al. [1998] supports the hypothesis that consumption of multivitamin supplementation before and during the early weeks of the pregnancy may protect against cleft lip with or without cleft palate. There was no significant evidence of an effect on CL/P risk attributable to the C677T *MTHFR* genotype, but the small differences in risk among the genotypes were in opposite directions for users versus nonusers of multivitamins, and could conceivably suggest a G×E interaction. It is well known that detection of G×E interactions can require very large sample sizes [Hwang et al., 1994; Andrieu and Goldstein, 1998], and so additional data will be required to definitively evaluate this possibility.

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TABLE I. Infant *MTHFR* Genotype, Maternal Use of Multivitamins Containing Folic Acid, and Risk (Odds Ratio) for Cleft Lip With or Without Cleft Palate Among All Race/Ethnic Groups*

MTHFR	Maternal vitamin use	No. of cases	No. of controls	Odds ratio	95% CI
CC	Yes	73	102	1.0	referent
CT	Yes	73	124	0.8	(0.5–1.2)
TT	Yes	19	36	0.7	(0.4–1.4)
CC	No	41	27	2.1	(1.2–3.8)
CT	No	36	30	1.7	(0.9–3.0)
TT	No	17	8	3.0	(1.2–7.2)

*Data from Shaw et al. [1998].

Recently, Mills et al. [1999] reported data indicating no significant differences in CL/P risk associated with the TT C677T *MTHFR* genotype, but unfortunately information about subjects' periconceptual maternal vitamin use (reported to be of very low rates in this population) was not available for incorporation into the analysis. Neither this study in Ireland nor Shaw et al.'s study in California have data to estimate folate consumption in the diet nor subjects' red cell folate levels. The Irish study did reveal a significant association of the TT genotype with isolated cleft palate (OR: 3.23, 95% CI: 1.32–7.86), thus encouraging further investigation of this SNP's effects on risk of oral clefts.

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